STUDIES OF HOMOLOGOUS AND HETEROLOGOUS TUMOR TRANSPLANTATION

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by

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INTRODUCTION

Doctor C. P. Rhoads, speaking at Yale Medical School this winter, ventured the opinion that current studies of host reactions to tumors were providing some of the most exciting leads in cancer research. Work at Dr. Rhoads' Sloan-Kettering Institute has shown that transplantation of cancer into people afflicted with terminal carcinomatosis will yield rapid and progressive growth of the implant while the same cancer transplanted into normal people will evoke an inflammatory response and subsequent death of the transplant. This demonstration of the altered reactivity of the person harboring a malignant growth points up the need to study the tumor host, his defenses and how they may be altered. The emphasis on the study of the host as well as the tumor has gained increasing recognition in the field of animal cancer research over the last decade.

THE ENCHANCING EFFECT

Woglom's review article, "Immunity to Transplantable Tumors", published in 1929 stressed the natural defenses to transplantation of tumors and the increased immunity to tumors following previous injection of that tumor (57). However, as early as 1907 Flexner and Jobling had found that rats injected with a heated emulsion of rat sarcoma ten days

prior to the injection of a live graft of this tumor had a greater number of growths and the tumors showed more rapid growth as compared to animals who had not received the heated emulsion (17). Unheated emulsion failed to reproduce this effect. Here, then, was evidence that prior contact with a tumor could decrease a rat's immunity to subsequent contact with the same tumor.

Casey (9,14) showed that prior injection of frozen Brown-Pearce rabbit tumor into the testicle, skin, subcutaneous tissue or muscle of rabbits changed these animals so that subsequent inoculations of live Brown-Pearce tumor gave increased number of growths, increased size of growths, decreased survival time and increased mortality as compared to animals which had not been pretreated. Pretreatment with fresh tumor did not elicit this phenomenon which Casey called the XYZ effect. The specificity of the XYZ effect was demonstrated by the inability of pretreatment with frozen Brown-Pearce tumor in mice to enhance the growth of Bashford mouse carcinoma (10). An even greater specificity of this reaction was revealed when Casey (11) showed that mouse sarcoma 180, anaerobically refrigerated for 14 to 60 days at 24° Fahrenheit, then minced, emulsified and used for pretreatment in mice enhanced the growth of grafts of live Sarcoma 180 but had no effect on the growth of grafts of Sarcoma 63

or Sarcoma 37. Material prepared from a uterine adenocarcinoma of rabbits did not enhance the growth of Brown-Pearce tumor (13). Furthermore, injection of 0.3 cubic centimeters of a filtrate of fresh Brown-Pearce tumor that had been passed through a Berkefeld V filter inhibited the growth of subsequent grafts of Brown-Pearce tumor (12).

Snell, Kaliss and their co-workers have studied intensively the effects of pretreatment of animals with lyophilized tissue, both normal and malignant. This pretreatment could enhance or inhibit the growth of subsequent live tumor transplants and which of the two predominated seemed, in large measure, to be determined by specific host strain-tumor relationship (49). Tumor 15091A, which grows in Strain A mice but not in Strains C57 or Balb C, when given as lyophilized pretreatment to the latter two strains induced an increase number of takes of subsequent grafts of live 15091A tumor. Lyophilized myeloid leukemia C1498 used as pretreatment in C57 leaden and C57 black strain mice caused a 50 per cent reduction in the number of takes of subsequent live C1498 grafts (48). Swiss white mice treated with frozen Sarcoma 180 then injected with viable tumor showed a decreased number of takes compared to animals that had not been pretreated (53). Kaliss and Snell (23) studied the growth of tumor 15091A in C57 black and C57 brown mice following pretreatment

with lyophilized normal tissue from Strain A mice, lyophilized tumor 15091A and lyophilized kidney from Strains C and P. The results are summarized below:

Pretreatment tissue	No. animals showing progressive growth Black	Brown
Strain A		
1. liver	8/31	2/10
2. kidney	13/30	6/10
3. spleen	9/14	9/10
4. red cells	0/24	-
Tumor 15091A	28/35	5/9
Strain C		
1. kidney	1/18	-
Strain P		
1. kidney	0/22	-
Controls	0/33	0/10

It was evident that lyophilized normal tissue from the natural host of the tumor could alter the resistence of a foreign strain to live grafts of that tumor (26). Kaliss (24) demonstrated that fresh tumor sediment could act to enhance tumor grafts. The enhancing effect of live tumor in Kaliss' system contrasts with the previously noted failure of Flexner and Jobling (17) to demonstrate an enhancing effect with live tumor.

The failure of non specific substances to produce the enhancing effect was shown by the inability of trypan

blue and lyophilized kidney, liver and spleen of the rat, hamster and guinea pig to enhance the growth of 15091A tumor in C57's (25,27). Other work, though, showed that normal kidney and spleen of C57 Bl mice used as lyophilized pretreatment in rats could enhance the take of a mouse mammary carcinoma in the rats although the mouse mammary carcinoma does not grow in the C57 Bl's (47). This suggestion of a species specificity is at odds with Casey's work with mouse sarcoma and the report of Kaliss and Snell, previously cited, that kidney from Strains C and P did not enhance the growth of tumor 15091A. This again points up the danger of generalizing beyond the specific host-tumor system being studied.

The use of cortisone with the pretreatment has been reported by Kaliss (33,36). Cortisone did not increase the number of takes and there is some suggestion that cortisone inhibits tumor graft growth when given in this manner.

The factors of age at onset of pretreatment and time interval between pretreatment and transplantation of live tissue has been studied. Kaliss found that pretreatment had to start before the live graft was made or, at the very latest, the day after the live transplantation (37). The effect of pretreatment is evident ten months after its conclusion in the mouse (32). Pikovski and Schlesinger (47) showed that the most important temporal factor was the age of the animal at the time pretreatment was begun. Animals

below ten days of age when pretreatment was begun gave consistently the highest number of takes. The time interval between pretreatment and the live grafting assumes much greater importance when live tissue rather than lyophilized tissue is used as pretreatment. If the time interval between two subcutaneous inoculations of Sarcoma I in C57Bl mice was greater than one month the second graft took while if the interval was less than one month the second graft regressed. None of the initial grafts grew (35).

The amount of lyophilized tumor used in the pretreatment is one determinate of whether the pretreatment will yield enhancement or inhibition. Mammary adenocarcinoma Eo771 injected into C3H Jax mice will produce a mortality rate of 50 per cent. Use of 50 milligrams of lyophilized Eo771 as pretreatment will greatly increase this mortality rate while use of .05 milligrams for pretreatment will decrease the mortality rate almost to zero. Dosages in between produce gradations of mortality which are directly proportional to the dose (22,30).

The use of specific antisera to transmit the enhancing effect has been elucidated by Kaliss and Molomut. In one study C57Bl/6Ks mice were injected with splenic tissue from Strain A mice and the serum from these C57's was injected into normal C57Bl/6Ks mice. The latter mice then received

transplants of Sarcoma I which is indigenous to Strain A. Six of fifteen animals so treated died while fifteen normal C57's received the grafting with no mortality (31). The same effect can be produced by antisera to Strain A kidney and to tumor 15091A which grows in Strain A (28). The per cent of animals with actively growing tumors after this method of pretreatment is much lower than that usually obtained after pretreatment with lyophilized tumor (28).

The growth of tumors in resistant animals following pretreatment of the animals produces a change in the tumor, as evidenced by an ability to grow in other normally resistent strains which have not been pretreated (34). A tumor from an inbred strain will grow in all mice of the same strain and F1 hybrids having that strain as one parent. Resistant backcross animals (F1 hybrids x resistant animal) have takes of between 50 per cent to .002 per cent with the tendency toward the lower figures. A tumor that grows in C3H's but not in C mice will, after growth in F1's of these two strains, when transplanted into resistant backcross mice yield three and one half times (30 per cent vs. eight per cent) greater mortality than those tumors received directly from C3H animals (2,4). This change in the tumor is permanent and can be repeatedly demonstrated after one passage through the resistant backcross, (3).

An indication that the change in host resistence following pre-treatment with lyophilized tissue has greater applicability than the host-tumor systems usually studied was revealed by an increased number of survivals of transplanted embryonic tissue and normal spleen in pretreated mice (29).

Snell (50) viewing the whole field of transplantation cited the factor of common ancestry as favoring the growth of homiotransplants (homiotransplants being defined as transplants from one inbred strain to another inbred strain). His study of the genetic pattern of transplantation pinpointed a "histocompatability gene" (H-2 allele) as being the determinate of the success or failure of a transplant (50).

The enhancing effect is basically immunological with the characteristics of (a) specificity, (b) persistency, (c) ability to be passively transferred, (d) necessity for pretreatment injections to be made before the period of active transplant growth (51).

The nature of the enhancing material in Sarcoma I from Strain A mice was delineated by Day, Kaliss and their co-workers (16). The enhancing factor was, (a) water extractable and water soluble, (b) unstable above 60° centigrade, (c) destroyed by prolonged autolysis or by acid and alkaline hydrolysis, (d) either a part of or associated with a denaturable high molecular weight protein, (e) in tissues it

appears to be a constituent of cellular particulates and presumably available only in small amounts as a circulating humoral factor.

ACTIVELY ACQUIRED TOLERANCE

Burnet and Fenner, in their book, "The Production of Antibodies", postulated that antibody production against a particular antigen can be specifically suppressed by exposure to the same antigen during embryonic life.

Such an exposure occurs in bovine twin embryos because of vascular anastomoses which allow admixture of the bloods of the twins (45). As adults these animals do not form antibodies to the blood cells of their twin. Experimentally producing an anastomosis between allantochorionic vessels of two chicks and then after birth challenging these chicks with their partner's erythrocytes reveals that these chicks do not form isoagglutins to the erythrocytes (19).

Burnet et.al. (8) inoculated chick embryos with 1) influenza, 2) bacterial viruses, and 3) human red cells in an attempt to prove experimentally their postulate. However, these animals as adults did not differ from control animals in their response to the three antigens.

In a classic work Billingham, Brent and Medawar (5) injected six 15 to 16 day old mouse embryos in utero with

a .01 milliliter suspension of tissue from the testes, kidney and spleen of adult A line male mice. The embryos were all part of one pregnancy of a CBA female. The injections were done at laparotomy and after the injections were completed the incision was closed and the pregnancy allowed to go to term. At that time five animals were born, there being no trace of the sixth. At age eight weeks the five animals were grafted with adult A line skin. Eleven days later two of these grafts were in an advanced stage of breakdown but the other three resembled autografts except for the strain specific albinism. Two of these three grafts were completely viable at the end of two months. That this was a specific reaction to A line skin was demonstrated when skin from another line was grafted to the animals with a resultant complete breakdown of this latter graft. The hosts ability to react to A line tissue had been altered but its reactivity to other antigens had not been altered. The initial grafts broke down when the hosts were inoculated with chopped lymph nodes from normal CBA's or from CBA's which had been immunized to A line skin (5,6). This proves that the antigenicity of the graft had not been impaired but rather that its take was dependent on changes in the host's resistence. The litters of these animals did not show any increased tolerance to A line skin (5).

At the sixteenth to seventeenth day of gestation ICR mouse embryos were injected with either (a) blood of C3H mice or (b) blood of F1 mice from C3H x DBA matings or (c) suspension of lymphosarcoma 6C3 which is lethel to C3H mice but does not grow in ICR mice. Six to eight weeks following birth the animals that had received the blood injections in utero were given intraperitoneal injections of 6C3HED which yielded an ascites tumor in all the animals (37). The embryos which received tumor cells had palpable tumors ten to 25 days after birth. These embryos were incapable of rejecting the tumor which is universally resisted by adult mice of the same strain. A suspension of the tumor grown in the embryonic mice injected into strains normally resistent to this tumor enjoyed a growth comparable to that in the indigenous strain. This loss of host specificity is similar to that previously noted by Barrett and Deringer (2).

Koprowski (38), elaborating on his earlier work, injected DBA mouselymphoma into ICR mouse embryos. This tumor does not grow in adult ICR mice but in this study takes of 75 to 100 per cent were reported in the embryos. This is another example of the embryos inability to produce resistence to a foreign antigen.

Wallace (56) was unable to produce tolerance to tumors when he injected rat embryos with tissue from the

strain that the tumor normally grows in. His injections were made on the sixteenth day of gestation, and the tissues used were normal cells of spleen, liver, kidney and testes suspended in a balanced salt solution. The following summarizes Wallace's results:

Tumor	Recipient	Antigen	No. with growth to death of host	
			Control	Test
1.C3HBA	Wistar	C3H cells	0/10	0/20
2.Sarcoma 180 (from C3H mice)	Wistar	C3H cells	0/9	0/21
3.dbrB (from DBA mice)	Wistar	DBA cells	0/17	0/16
4.35FM (from Sprague-Dawley rats)	Hooded	Sprague-Dawley cells	0/15	0/17
5.53FM (from Sprague-Dawley rats)	Hooded	Sprague-Dawley cells	0/15	0/7
6.53FM (from Sprague-Dawley rats)	Wistar	Sprague-Dawley cells	0/20	4/18

Wallace suggests that the use of the same tumor for treatment of the embryos and for the adult graft might have produced better results. Billingham et. al., though, achieved their success through the use of normal tissue for pretreatment. Here again the difference may lie in a species specificity or a specificity within a species. The latter is suggested by Wallace's limited success with one host-tumor system (53FM tumor-Wistar rats).

Toolan (55) was also unsuccessful when she tried to produce tolerance in rats to human tumors by in utero

injections. Her pretreatment material was normal human tissue which was obtained from persons other than those who yielded the carcinoma. This difference in the antigen and the tumor may, in part, explain the failure.

Felton (16) found that injection of 0.5 milligrams of soluble polysaccharide into C3H mice paralyzed the animals ability to produce antibody to injected pneumococci.

The susceptibility of the mouse embryo to treatment with foreign antigen as shown by Billingham et. al. raises the question of whether the embryo can be treated by ways other than direct injection. The transmission from mother to fetus of passive immunity to certain diseases has long been recognized. This illustration of the passage of antibodies through the placenta has been supplemented by work showing that many drugs pass from mother to child in utero, e.g. barbiturates, narcotics (18). Studies of erythroblastosis fetalis gave rise to the belief that fetal red cells pass into the maternal circulation and thus stimulate antibody formation (39). This transplacental transfer of red cells was believed to be on a limited scale and due to imperfections of the placenta peculiar to this disease. That this admixture of blood cells occurs in normal pregnancies and through normal placentas was conclusively demonstrated by Mengert and his group (41). They injected normal

pregnant women with sickle cells and at birth recovered these sickle cells from cord blood.

In this study it was hoped that by injecting pregnant mice with a subcutaneous tumor a change in the reactivity of the embryos to that tumor could be elicited. This could be accomplished in one of two ways. Firstly, tumor cells from the mother might pass to the embryo. If red cells can pass through a human placenta it is not too unreasonable to hope that tumor cells could pass through the less complex mouse placenta. Secondly, the formation by the mother of specific antiserumto the tumor with subsequent transmission to the fetus of that antiserum might enhance the growth of the tumor in the fetus. The use of such antisera to enhance tumor growth has previously been cited in the discussion of the work of Kaliss and Molomut (28). Or, more directly, enhancing factor itself might be transmitted through the placenta.

METHODS AND MATERIALS

This study was divided into two parts.

PART ONE

Pregnant mice of Line CB_2-j were inoculated subcutaneously in the axillary region with live mouse fibrosarcoma 397. This tumor does not grow in CB-j mice. The animals in this experiment were two to four months old. The tumor was obtained from BC mice carrying the tumor which was in its nineteenth to twentyfourth generation.

The offspring from these pregnancies received subcutaneously in the interscapular area inoculations of tumor 397 at age eight to nine days. These animals were checked for tumor growth four to five times weekly until they were six weeks of age.

PART TWO

Pregnant animals of the BC-a, BC-b, BC-c and BC-f lines were inoculated subcutaneously in the axillary region with live mouse fibrosarcoma 397. This tumor grows progressively in these strains producing very large ulcerating lesions which do not metastasize but which kill 90 to 100 per cent of the mice within four weeks. The mice in this experiment were two to four months old. The tumor was obtained from BC mice carrying the tumor which was in its 19-24 generation.

The offspring from these pregnancies were inoculated subcutaneously in the interscapular area with tumor 397 within 24 hours of birth. These animals were checked for tumor growth four to five times per week. All animals were maintained on a prepared diet (Purina Laboratory Chow) and water ad libitum.

BC refers to a group of mice derived by hybridizing PM females and C3H males and backcrosses to the PM mice followed by brother-to-sister inbreeding. Eleven sublines were established and designated by lettering from a to k. The mice used in the study have been inbred brother-to-sister for more than 25 generations since the original hybridization.

CB_2-j refers to hybrid mice derived by mating BC-j females with C57 males.

Fibrosarcoma 397 is a tumor that arose in a BC30 female (30 here refers to the number of generations of inbreeding) which starting at age 72 days had received 1.25 milligram weekly of testosterone proprionate. Between day 72 and 78 this animal also received x-radiation in three exposures of 154r each on alternate days. At death at age 615 days this animal had a large sarcoma at the injection site covering 2x3 centimeters of the lower back. There was a 14x11 millimeter tumor growth in the liver and numerous smaller implants in this organ. The kidneys and spleen were normal. The adrenals were large and spotted. Left ovary was 2x3

millimeter and there was a possibility that it contained tumor. The right ovary was very small. The uterus was well developed. The pituitary was normal.

This tumor which originated in January of 1956 has been maintained by passage in BC sublines a, b, c, d, f and now (April 1957) is in its twenty seventh generation of serial transfer. The tumor does not grow in BC sublines g and j. Subcutaneous inoculation of the tumor into susceptible animals yields large ulcerating lesions without metastases which kill 90 to 100 per cent of the animals within three to four weeks.

In another aspect of this study tissue from human uterine myomata was transplanted into the anterior chamber of the eyes of mice and guinea pigs (20,42). These animals had been inoculated subcutaneously with pellets containing diethylstilbestrol (25 per cent) and cholesterol (75 per cent) (40). The tissue was obtained at laparotomy and, through the cooperation of the surgeons, was removed before the blood supply of the uterus was compromised.

One group of mice received injections of .25 milligrams of prednisolone every other day for a period of two weeks starting on the day before transplantation. This was in addition to the pellets of diethylstilbestrol-cholesterol.

OBSERVATIONS

The inoculation of tumor into susceptible BC animals within the first 24 hours after birth provoked an extreme degree of cannibalism by the mothers. It is conservatively estimated that over 100 animals were lost to follow up because of this infanticide. Most of the cannibalism occurred within 24 hours of the inoculation but the survivors of this period were jeopordized again between ten and twelve days of age. Only three animals with tumors survived past day 13. Therefore, it was apparent that the growth of the tumors had to be ascertained at day ten in order to obtain any sort of sample.

All of the BC mothers with tumors bore large ulcerating lesions which killed them in three to four weeks. There was no evidence of metastases.

All matings in the study of BC animals were between litter mates.

The tissue from the human uterine myomata was obtained from three different patients, all of whom were undergoing total abdominal hysterectomy. Though grossly the transplants appeared to become vascularized after six or seven days in the mice and guinea pigs, histological section of the tissue between day 12 to 21 revealed only necrotic tissue suggestive of muscle, or fibrous tissue hyperplasia. The

latter was interpreted as arising from the host animal in response to the foreign body. In the group of mice receiving cortisone the eyes rapidly became infected and eventually became abscesses. There was no trace of the transplants in these animals after three weeks.

RESULTS

TABLE I

Growth of Tumor 397 in Mice Born to CB_2-j Mothers Carrying This Tumor

Mother	Day of Gestation Mother Inoculated	Litter	Age at Grafting	No. with Tumor/Litter
CB_2-j ♀ 135BC38	16	Males: 1 brown, 1 black Females: 5 black, 1 white	9 days	0/8*
CB_2-j ♀ 33BC38	16	Males: 3 black, 1 brown Females: 1 black, 1 brown, 1 white	9 days	1/9
CB_2-j ♀ 135BC35	19	Males: 1 black, 3 white Females: 2 black, 2 brown, 1 white	8 days	0/9
CB_2-j ♀ 33BC38	No inoculation	Males: 3 black Females: 3 black, 1 white	9 days	0/7
CB_2-j ♀ 133BC35	No inoculation	Males: None Females: 8 black	9 days	0/8
CB_2-j ♀ 133BC35	No inoculation	Males: 2 black Females: 1 black, 1 brown, 1 white	9 days	1/5

*Three of these animals had subcutaneous abcesses measuring between three and six millimeters in diameter which were originally mistaken for tumefactions.

TABLE II

Growth of Tumor 397 in Mice Born to BC Mothers Carrying this Tumor

Mother	Day of Gestation Mother Inoculated	No. in Litter	Survivors at 10 days	Size of Tumors
BC37b 113BC36	17	8	3	5 mm 5 mm 4 mm
BC38f 2BC37	16	9	2	6 mm no growth
BC37c 61Bc36	13	10	1	5 mm
BC36b 147BC35	17	7	2	5 mm 4 mm
BC37d 52BC36	13	6	2	4 mm no growth
BC36b 161BC35	Mother not inoculated	8	2	5 mm* 4 mm
Bc38b 74Bc37	Mother not inoculated	8	1	5 mm

*This animal survived to the age of 22 days at which time his tumor covered most of the back and measured over 16 millimeters in length.

There was no tumor growth in any of the CB2-j mothers. The BC mothers with tumor implants all had progressive growth of their tumor and these animals were dead within three to four weeks after implantation of the tumor.

The one tumor that grew in an experimental mouse of CB2-j parentage was in a black male. This tumor was densely adherent to the skin and was bilobar. The animal was sacrificed at the age of 22 days (13 days after tumor implantation) at which time the two lobes of the tumor measured five millimeters and three millimeters in diameter respectively. Autopsy failed to reveal any metastases. A white female control mouse of CB2-j parentage had a four millimeter in diameter subcutaneous tumor when sacrificed at 22 days of age. There were no metastases.

There is no statistical difference between the rate of growth in the experimental and in the control BC mice. The data is summarized in Table II.

DISCUSSION

The failure to demonstrate an altered reactivity to fibrosarcoma 397 in animals born to mothers who carried this tumor subcutaneously may be explained in two parts, each part related to one of the hypotheses upon which this study was based.

The first hypothesis was that tumor cells from the mother could pass through the placenta into the fetus. Koprowski (38) showed that tumors inoculated into embryos would yield progressive growth. However, in this study susceptible BC embryos never showed evidence of metastatic disease which makes it unlikely that maternal tumor cells ever reached the fetus. This possibility cannot be entirely ruled out since it has been demonstrated that tumor cells free in the peritoneal cavity of humans do not always give rise to metastatic growths.

Fibrosarcoma 397 though a highly vascular tumor does not usually metastasize. In retrospect the choice of this tumor for the study was an unfortunate one. Even with a metastasizing tumor the possibility of tumor cells reaching the placenta would be limited by mechanical factors (15). Clinical reports of placental metastases are extremely rare (1).

The second hypothesis was that immune sera from the mother would pass to the fetus and act as an enhancing factor. Another possibility was that enhancing factor itself would pass to the fetus. This study would seem to indicate that no such thing occurred.

Fibrosarcoma 397 may not produce an enhancing factor in the host animals or in any host.

One early study suggested that live tumor did not contain enhancing factor in useable form and that physical alteration, e.g., freezing, heating, was necessary to elicit the enhancing factor (17). No such physical alteration was used in this study. However, other studies have successfully used live tissue, (35,26).

Kaliss (35) gave two subcutaneous inoculations of live Sarcoma I to resistent C57Bl/6ks mice and found that if the inoculations were separated by an interval of greater than one month the grafts took while if the interval was less than one month the second grafts regressed. None of the initial grafts in the study took. In all our own experiments the interval between inoculation of the mother and the subsequent inoculation of the offspring was less than a month.

The need in clinical medicine for a method of transplanting tissue such as skin or an organ like the kidney has

led to many attempts at homotransplantation. With the exception of transplants between identical twins or into agammaglobulinemic subjects these homotransplants have been universally unsuccessful (7,44). The possibility that treatment of a pregnant woman could alter the response of her offspring to foreign tissued or organs in such a way that the tissued would grow in the offspring is an intriguing one. The failure of this study to demonstrate such an effect in mice with fibrosarcoma 397 should not discourage further research along these lines.

CONCLUSION

There was no evidence that animals born to mothers who had been inoculated with mouse fibrosarcoma 397 had an altered reactivity to injection of that tumor.

SUMMARY

1. The experimental work relating to the effect of altered host resistance to transplants is reviewed under the headings of "Enhancing Effect" and "Acquired Tolerance".

2. Pregnant animals of strains both resistant and susceptible to mouse fibrosarcoma 397 were inoculated with this tumor. The offspring of these pregnant animals were inoculated in the neonatal period with fibrosarcoma 397.

3. There was no difference in the growth of the tumor in these offspring as compared to the offspring of mothers which had not been inoculated with the tumor.

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Figure 1: Fibrosarcoma 397 from BC mouse of this study. Spindle cells arranged in interesting whorls and strands; no necrosis; abundant capillery and sinusoidal blood supply; minimal amounts of collagen. Amazingly few mitoses for such a rapid growing tumor. H. and E. stain (x100).

Figure 2: Fibrosarcoma 397 from first generation transplant of this tumor. Many more mitotic figures than that seen in later generations of the tumor used in this study. Area showing invasion of muscle fibers. H. and E. stain (x200).

Figure 3: Another section from first generation transplant. H. and E. stain.

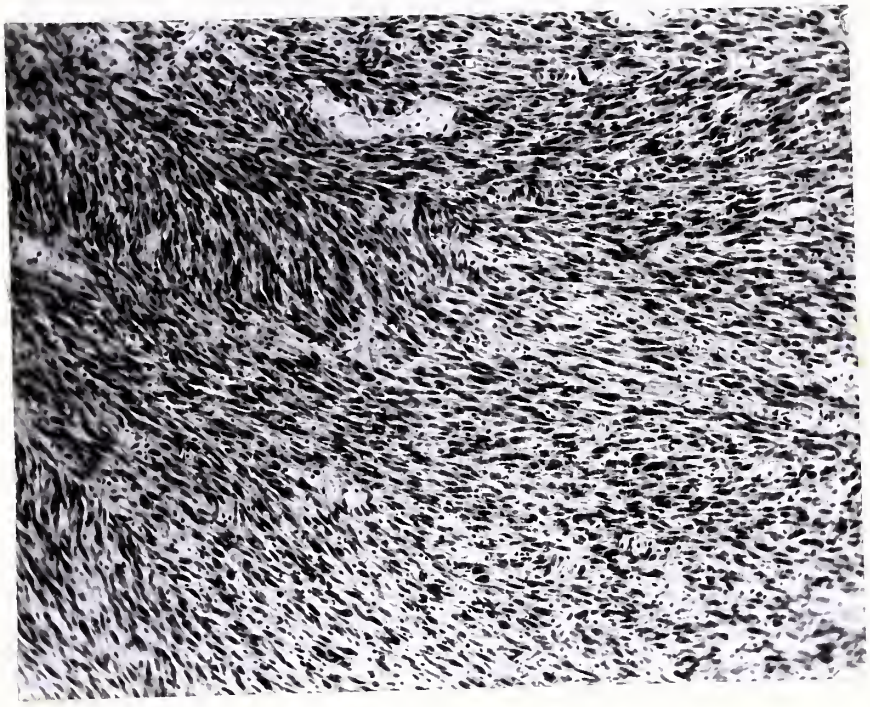

Figure 1

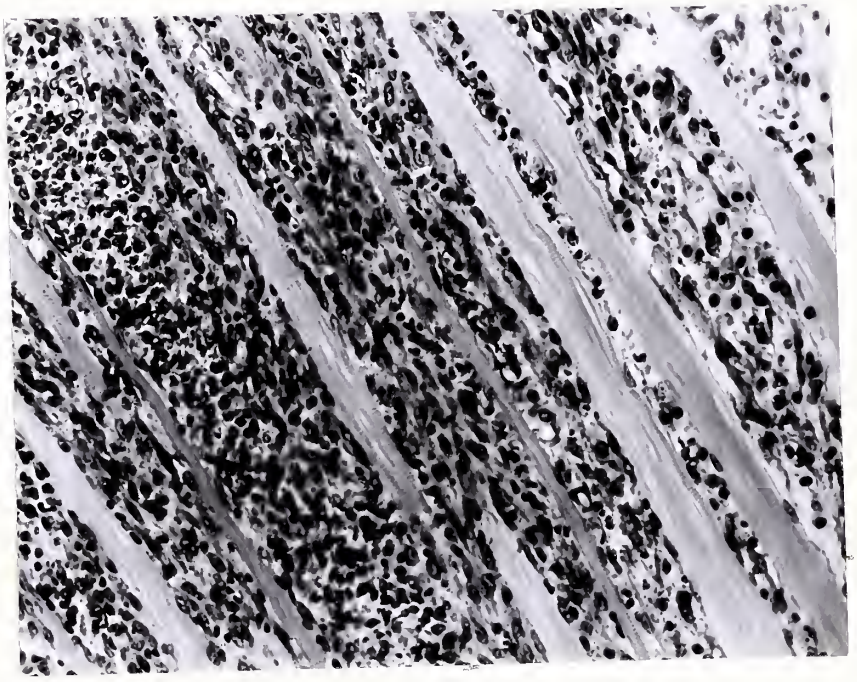

Figure 2

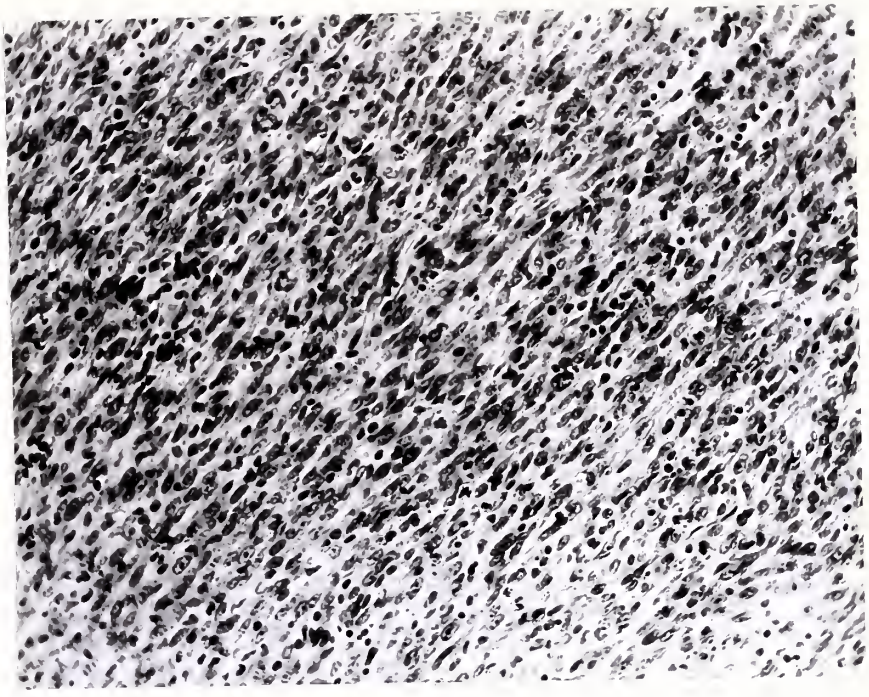

Figure 3